

- (c) applying ultrasound to the subject;
- (d) obtaining an ultrasound image of the left ventricle of the heart of the

subject.

99. (New) The method of claim 98 wherein said lecithin is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

100. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

- (a) suspending hydrogenated soya lecithin, dicetylphosphate and lactose in solution in the presence of  $\text{CF}_4$  gas to form a gas-filled microbubble suspension;
- (b) administering portions of the gas-filled microbubble suspension to the subject;
- (c) applying ultrasound to the subject;
- (d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

101. (New) The method of claim 100 wherein said lecithin is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

102. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

- (a) suspending hydrogenated soya lecithin, dicetylphosphate and lactose in solution in the presence of  $\text{C}_4\text{F}_{10}$  gas to form a gas-filled microbubble suspension;
- (b) administering portions of the gas-filled microbubble suspension to the subject;
- (c) applying ultrasound to the subject;

(d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

103. (New) The method of claim 102 wherein said lecithin is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

104. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

(a) suspending hydrogenated soya lecithin, dicetylphosphate and lactose in solution in the presence of a physiologically compatible gas to form a gas-filled microbubble suspension, said gas selected from the group consisting of  $\text{CF}_4$ ,  $\text{C}_4\text{F}_8$  and  $\text{C}_4\text{F}_{10}$ ;

(b) administering portions of the gas-filled microbubble suspension to the subject;

(c) applying ultrasound to the subject;

(d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

105. (New) The method of claim 104 wherein said lecithin is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

106. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

(a) suspending dipalmitoylphosphatidyl glycerol, a copolymer of polyoxyethylene-polyoxypropylene with a molecular weight of 8400 and glycerol in solution in the presence of  $\text{C}_4\text{F}_8$  gas to form a gas-filled microbubble suspension;

(b) administering portions of the gas-filled microbubble suspension to the subject;

- (c) applying ultrasound to the subject;
- (d) obtaining an ultrasound image of the left ventricle of the heart of the

subject.

107. (New) The method of claim 106 wherein said dipalmitoylphosphatidyl glycerol is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

108. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

- (a) suspending dipalmitoylphosphatidyl glycerol, a copolymer of polyoxyethylene-polyoxypropylene with a molecular weight of 8400 and glycerol in solution in the presence of  $\text{CF}_4$  gas to form a gas-filled microbubble suspension;

- (b) administering portions of the gas-filled microbubble suspension to the subject;

- (c) applying ultrasound to the subject;

- (d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

109. (New) The method of claim 108 wherein said dipalmitoylphosphatidyl glycerol is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

110. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

- (a) suspending dipalmitoylphosphatidyl glycerol, a copolymer of polyoxyethylene-polyoxypropylene with a molecular weight of 8400 and glycerol in solution in the presence of  $\text{C}_4\text{F}_{10}$  gas to form a gas-filled microbubble suspension;

(b) administering portions of the gas-filled microbubble suspension to the subject;

(c) applying ultrasound to the subject;

(d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

111. (New) The method of claim 110 wherein said dipalmitoylphosphatidyl glycerol is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

112. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

(a) suspending dipalmitoylphosphatidyl glycerol, a copolymer of polyoxyethylene-polyoxypropylene with a molecular weight of 8400 and glycerol in solution in the presence of a physiologically compatible gas to form a gas-filled microbubble suspension, said gas selected from the group consisting of  $\text{CF}_4$ ,  $\text{C}_4\text{F}_8$  and  $\text{C}_4\text{F}_{10}$ ;

(b) administering portions of the gas-filled microbubble suspension to the subject;

(c) applying ultrasound to the subject;

(d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

113. (New) The method of claim 112 wherein said dipalmitoylphosphatidyl glycerol is in lamellar or laminar form and stabilizes the microbubble boundary at the gas to liquid interface.

114. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

(a) sonicating human serum albumin in solution in the presence of CF<sub>4</sub> gas to form a microballoon suspension which microballoons are bounded by a material envelope formed with the albumin and are filled with the gas;

(b) administering portions of the microballoon suspension to the subject;

(c) applying ultrasound to the subject;

(d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

115. (New) A method of ultrasound imaging of the left ventricle of the heart of a subject consisting of:

(a) sonicating human serum albumin in solution in the presence of a physiologically compatible gas to form a gas-filled microballoon suspension, said gas selected from the group consisting of CF<sub>4</sub>, C<sub>4</sub>F<sub>8</sub> and C<sub>4</sub>F<sub>10</sub> and which microballoons are bounded by a material envelope formed with the albumin and are filled with the gas;

(b) administering portions of the microballoon suspension to the subject;

(c) applying ultrasound to the subject;

(d) obtaining an ultrasound image of the left ventricle of the heart of the subject.

### REMARKS

Claims 50-97 were pending in this application. New claims 98-115 are added with this amendment.

No new matter has been added. Support for each of the new claims 98-115 are found throughout the specification and original claims as filed. For example, the subject matter of claims 98-105 are disclosed, *inter alia*, in Examples 4 (in vitro testing) and 5 (in vivo testing)

and the subject matter of claims 106-113 are disclosed, *inter alia*, in Example 8. The subject matter of claim 114-115 are closed, *inter alia*, in Example 1 and Example 7.

Applicants are not aware of any prior art that has all of the elements of the claims or which in proper combination with other prior art would provide all of the elements of the claims. For example, Applicants are not aware of any prior art or any combination thereof which teaches or suggests the elements of claims 98-105 such as, but not limited to: (a) suspending hydrogenated soya lecithin, dicetylphosphate and a lactose in solution in the presence of the claimed gas and (d) obtaining an image of the left ventricle of the heart of the subject.

Similarly, Applicants are also not aware of any prior art or any combination thereof which teaches or suggests the elements of claims 106-113 such as, but not limited to: (a) suspending dipalmitoylphosphatidyl glycerol, a copolymer of polyoxyethylene-polyoxypropylene with a molecular weight of 8400 and glycerol in the presence of the claimed gas; and (d) obtaining an image of the left ventricle of the heart of the subject.

Furthermore, Applicants are not aware of any prior art of combinations thereof which teaches or suggests the additional element of claims 99, 101, 103, 105, 107, 109, 111, and 113 wherein the lecithin or dipalmitoylphosphatidyl glycerol is in lamellar or laminar form and stabilize the microbubble boundary at the gas to liquid interface.

Nor are Applicants aware of any prior art or any combination thereof which teaches or suggests the elements of claims 114-115 such as, but not limited to: (a) sonicating human serum albumin in solution in the presence of the claimed gas to form a gas filled microballoon suspension in which the microballoons are bounded by a material envelope formed with the albumin and are filled with the gas and (d) obtaining an image of the left ventricle of the heart of the subject.

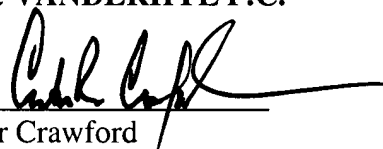
Thus, as these new claims 98-115 are fully supported in the specification and are fully patentable over any references cited, favorable action on these claims is requested.

If there are additional fees, please charge them to our firm Deposit Account No. 14-1140.

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

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